Original Article

Polymorphisms of XPC Gene And Susceptibility of Esophageal Cancer

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ABSTRACT

Objective: To explore the relationships of the polymorphisms of xeroderma pigmentosum C (XPC) and the susceptibility of esophageal cancer (EC) in Changzhi area, China.

Methods: The study was conducted by a case-control study which included 196 cases of EC and 201 cases of controls. XPC PAT polymorphisms were determined with polymerase chain-restriction on fragment length polymorphism (PCR-RFLP).

Results: The frequencies of wild homozygote (PAT−/−), mutation heterozygote (PAT−/+) and mutation homozygote (PAT+/+) of XPC were 36.73%, 51.53% and 11.74% in case group, 37.81%, 52.24% and 9.95% in control group, respectively, and there was no significant difference between the two groups ($\chi 2$ =0.332, P=0.847). There was no interaction between XPC PAT mutation genotype and xeroderma pigmentosum A (XPA) (S=0.85) and pickled food (S=0.81).

Conclusion: A genetic polymorphism in XPC may be not associated with esophageal cancer in Changzhi population.

Key words: Esophageal Cancer; XPC PAT; Genetic polymorphisms

INTRODUCTION

Esophageal Carcinoma (EC) is one of the world's most common malignant tumors. The number of death was 440,000 in the year of 2002 in the world, based on the WHO Health Report 2004. China has the world's highest adjusted mortality rate (23.40/100,000). It is not clear that the cause of esophageal carcinoma up to now. Researchers indicate that alteration of DNA repair genes in general population plays an important role in genetic susceptibility to cancer. DNA repair may lead to increased risk of cancer. As a defense mechanism of cell, nucleotide excision repair (NER) is the key player in removing bulky DNA adducts and maintaining genome stability. The NER pathway has been extensively studied and the main component genes involved in human NER have been elucidated[1, 2]. Xeroderma pigmentosum group C (XPC), which is a protein related to human disease xeroderma pigmentosum, is involved in the damage recognition step in nucleotide excision repair[3]. Genetic variations in XPC gene might be associated with altered DNA repair capacities (DRC). The ability to monitor and repair carcinogen-induced DNA damage is an important determinant of susceptibility to carcinogenesis[4]. The XPC gene, which encodes a 940 amino acid protein uniquely involved in global genome repair, spans 33 kb, and has 16 exons (82–882 bp) and 15 introns (0.08–5.4 kb)[5]. Two most common polymorphisms [Lys939Gln (XPC A33512C, rs2228001) and a poly (AT) insertion/deletion polymorphism (XPC PAT −/+) in intron 9] in XPC family are associated with risks of many human malignancies[6]. The PAT+ polymorphism is associated with increased risks of skin, head and neck, and esophageal cancer[7–9]. The variant allele of

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Lys939Gln (A to C) may be also associated with increased risks of skin, breast, and bladder cancers[7, 10, 11]. However, the related studies on these polymorphisms in cancer have shown conflicting results.

Changzhi area, located in the Taihang Mountain area, is one of China's high-incidence areas of esophageal carcinoma. Thus, the present study was undertaken to access the genetic polymorphisms of XPC and susceptibility to esophageal cancer in changzhi area.

MATERIALS AND METHODS

Study Population

One hundred and ninety-six patients with esophageal carcinoma and two hundred and one controls were enrolled in the study. The cases and controls were matched by sex and age (±5 y), collected from two medical centers in Changzhi from August 2003 to July 2006. The cases were correctly diagnosed by gastroscope and pathology means, and did not have any else tumor. The controls had no tumor history and relative disease of esophagus in the same stage.

A Questionnaire Survey

The survey included general demographic characteristics, eating habits, living patterns of behavior (such as cigarette smoking, alcohol drinking), trauma history, family history of esophageal carcinoma. The variables had clear definition in order to reduce the selection and information bias. We conducted the investigation by trained investigators.

Genotyping of XPC[12]

Each patient provided 2 ml pre-treatment blood for the study. The blood samples were collected in EDTA citric acid anticoagulation tubes and stored at -80°C until analysis. Genomic DNA was isolated from the blood samples using the DNA purification kit or phenol chloroform extract, and stored at 4°C until use. XPC genotypes were determined by a PCR-RFLP assay. The PCR primers for the PAT polymorphism were 5'-TAGCACCCAGCAGTCAAAG, and 5'-T-GTGAATGTGCTTAATGCTG-3'. Amplification was done with an initial denaturation at 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, with a final extension step at 72°C for 10 min. Next, 5 μl of the PCR product was resolved for 30 min at 220 V on 3% agarose gel stained with ethidium bromide.

Statistical Analysis

χ^2-test was used to test the differences in the distributions of qualitative variables. Hardy-Weinberg equilibrium was tested to compare the observed genotype frequencies to the expected genotype frequencies among the subjects. Analyses of the risk factors were conducted using univariate and multi-variables nonconditional logistic regression. All *P* values were two-tailed and at α=0.05 level. All the data were analyzed by SPSS13.0 statistical software.

Interaction Analysis

Using the additive model, the index included the synergy index S, when S=1, there is not interaction between factors, when S>1, there is synergistic interaction between factors, when S<1, there is negative interaction between factors, attributable proportion of interaction (API) and relative excess risk of interaction (RERI).

It is here that $S = (OR_{11} - 1) / (OR_{01} - 1) + (OR_{10} - 1)$; $API = [OR_{11} - (OR_{10} + OR_{01}) + 1] / OR_{11}$; $RERI = OR_{11} - (OR_{10} + OR_{01}) + 1$.

RESULTS

Characteristics of Study Population

A total of 196 esophageal carcinoma cases and 201 control subjects were included in this study. This analysis included 125 male (63.78%) and 71 female (36.22%) subjects aged 38−82 years (mean age, 59.09±8.43) in cases, 130 male (64.68%) and 71 female (35.32%) subjects aged 37−80 years (mean age, 58.81±8.58) in controls. The sex ratio, in two groups, was 1.76:1 and 1.83:1 respectively. There were no significant differences in the distribution of age and sex between cases and controls.

The Results of Nonconditional Logistic Regression Analysis

The study indicated that thirteen factors were correlated with EC ($P<0.05$) in the level (α=0.05) by univariate nonconditional logistic regression analysis. By multi-variables nonconditional logistic regression analysis, it showed that food with more meat and egg was a guarding factor to EC, and other seven factors were risk factors of esophageal cancer in Changzhi area, including hard diet and pickled food, quick eating, alcohol drinking, cigarette smoking, fierce

mind stimulation, and family history of esophageal carcinoma (Table 1).

Hardy-Weinberg Equilibrium Test

The frequency distribution of the XPC genotypes (PAT–/–, PAT–/+, PAT+/+) in controls was 76,105 and 20, respectively. The distribution of the different genotypes among the 201 controls closely conformed to expected Hardy-Weinberg frequencies (χ^2=3.54, P>0.05).

The Results of XPC Genotyping

The primers for XPC intron 9 generated a single band representing the entire 266 bp fragment (the wild-type or PAT– alleles) or a 344 bp fragment (the polymorphic or PAT+ alleles), PAT–/+ heterozygotes yielded both bands (266 bp and 344 bp fragments) by PCR (Figure 1).

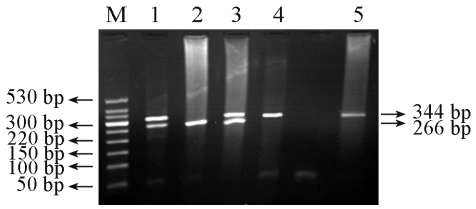

Figure 1. PCR results of XPC genotyping. Lanes 1, 3: Heterozygosity; Lane 2: Wild type; Lanes 4, 5: Mutation type

Table 1. Non-conditional logistic regression analysis of risk factors to esophageal cancer

Factors	β	$x\pm s$	Wald χ^2	P	OR	95% CI
Meals and eggs food	–0.98	0.38	6.65	0.01	0.38	0.18–0.79
Liking to eat hard diet	0.83	0.25	10.95	0.00	2.29	1.40–3.75
Pickled food	0.88	0.29	8.82	0.00	2.40	1.35–4.27
Meal quickly	0.59	0.25	5.50	0.02	1.80	1.10–2.94
Alcohol drinking	0.88	0.31	7.97	0.01	2.40	1.31–4.41
Cigarette smoking	0.19	0.07	7.58	0.01	1.21	1.05–1.39
Fierce mind stimulation	0.97	0.27	12.54	0.00	2.63	1.54–4.50
Family history of EC	0.84	0.40	4.39	0.04	2.32	1.06–5.08

OR: Odd ratio; 95% CI: 95% confidence interval.

Table 2. Relationship between the distribution of XPC genotype and esophageal cancer

XPC	Cases n (%)	Controls n (%)	OR (95% CI)	χ^2	P
Allele					
PAT-	245 (62.50)	257 (63.93)	1.06 (0.79–1.42)	0.175	
PAT+	147 (37.50)	145 (36.07)			
Genotypes					
PAT-/-	72 (36.73)	76 (37.81)	1.00		
PAT-/+	101 (51.53)	105 (52.24)	1.01 (0.67–1.55)	0.005	0.944
PAT+/+	23 (11.74)	20 (9.95)	1.21 (0.18–0.56)	0.312	0.576

χ^2=0.332; P=0.847; OR: Odd ratio; 95% CI: 95% confidence interval.

Distribution of Different XPC Genotypes and the Relationship between the XPC Polymorphism and Esophageal Cancer

The result indicated that distribution of allele gene of XPC had no significant association between cases and controls group (P>0.005). The frequencies of wild homozygote (PAT–/–), mutation heterozygote (PAT–/+) and mutation homozygote (PAT+/+) of XPC were 36.73%, 51.53% and 11.74% in case group,

37.81%, 52.24% and 9.95% in control group, respectively, and there was no significant difference between the two groups (χ^2=0.332, *P*=0.847) (Table 2).

Interaction

The details of interaction analysis between XPC PAT genotyping and polymorphisms of xeroderma pigmentosum A (XPA) in this study are shown in Table 3. The results showed that there was no interaction in individuals with XPC PAT genotype and XPA genetype to esophageal cancer risk (S=0.85). Also, the data, in Table 4, indicated that there was no interaction between XPC PAT genotyping and eating pickled foods to esophageal cancer risk (S=0.81).

Table 3. Interaction between XPC genotype and XPA genotype

XPC	XPA	Cases	Controls	OR	95% CI	*P*
0	0	17	10	1.03		
0	1	55	74	0.44	0.19–1.03	0.054
1	0	68	44	0.91	0.38–2.17	0.830
1	1	56	73	0.45	0.19–1.06	0.062

1 means exposure; 0 means noexposure; OR: Odd ratio; 95% CI: 95% confidence interval.

Table 4. Interaction between XPC genotype and pickled food

XPC	Pickled food	Cases	Controls	OR	95% CI
0	0	62	79	1.00	
0	1	43	32	1.71	0.97–3.01
1	0	52	60	1.10	0.67–1.82
1	1	39	30	1.66	0.93–2.96

1 means exposure; 0 means noexposure; OR: Odd ratio; 95% CI: 95% confidence interval.

DISCUSSION

XPC is one kind of conservative evolution of the DNA repair enzyme in nucleus. In the nucleotide excision repair pathway, XPC can repair the damaged DNA together with other proteins. Hence, any variations that may occur in XPC gene might have potential to affect protein function and subsequently DRC[13]. Some studies have found that the PAT polymorphism might increase the risk of lung cancer and head and neck cancer. In a hospital-based case-control study, Marin[14] indicated that XPC PAT+/+ might contribute to the risk of developing lung cancer in the Spanish population (OR=1.60; 95% CI:1.01–2.55). Shen[8] reported that XPC PAT+/+ subjects were at significantly increased risk for head and neck cancers (OR=1.85, 95% CI:1.12–3.05). A study suggested that bladder cancer is associated with early onset of age in XPC PAT+/+ (*P*=0.021, OR=2.49). On the basis of age stratification, the patients carrying the XPC +/+ genotype with age <60 (*P*=0.04, OR=3.58) were observed to be at 3-folds elevated risk of bladder cancer[15]. But there were some different research reports about the association between the XPC polymorphism and cancer in different results. A meta-analysis found that the XPC PAT polymorphism might decrease cancer risk associated with the PAT+/– genotype only in Asians compared with the PAT–/– genotype[16]. Other study showed that XPC had no association with risk of breast cancer[17]. Homozygous carriers of the PAT+ allele had lower DNA repair capacity than homozygous carriers of the PAT– allele on lymphocytes from 102 healthy subjects[18].

For further study, we conducted the study to explore the relationship between XPC polymorphisms and EC susceptibility. The finding showed that there was no significantly difference between controls and esophageal cancer patients. Individuals carrying XPC PAT+/+ genotype was no at increased risk for esophageal cancer as compared with those mutation heterozygote (PAT–/+) or wild homozygote (PAT–/–) genotype. The results indicated that there was no relationship between XPC polymorphisms and

esophageal cancer susceptibility. Our present study is different to the research conducted by Casson AG, who reported that patients with esophageal adenocarcinoma (EADC) demonstrated a significantly higher frequency of the XPC PAT homozygous variant genotype compared with asymptomatic controls (OR=3.82; 95% CI=1.05–13.93)[19].

The interaction of XPC genotypes with XPA and pickled food has also analyzed to study the modulation of esophageal cancer risk in our investigation. There were not interaction between XPC genotype and XPA genotype and pickled food to esophageal cancer risk. Also, no interaction between XPC genotype and alcohol drinking and cigarette smoking exposure was showed. Our study only included 196 esophageal cancer cases and 201 controls, and is thus a small study group. We cannot exclude that our findings are due to chance, but the result may still contribute to meta-analysis. This is the first case-control study of XPC polymorphisms in relation to esophageal cancer in this area.

Our study observed that liking to eat hard diet and pickled food, meal quickly, alcohol drinking, cigarette smoking, fierce mind stimulation, and family history of esophageal carcinoma were the main risk factors of esophageal cancer in this area. It indicates that ill eating habits, behavior patterns, and family history of esophageal carcinoma are closely related to the occurrence of esophageal cancer. Other studies also showed similar results[20, 21].

In conclusion, our findings have shown that the XPC PAT genotype is not associated with the risk for developing esophageal cancer in the population of Changzhi. It is necessary for further study to validate the functional polymorphic effect of the XPC gene on esophageal cancer in a large population. Therefore, at present, it is important to prevent and early treat esophageal cancer for advocating a healthy lifestyle, changing unhealthy habits, maintaining good interpersonal relationships, and revealing the relationship between gene polymorphism and environmental factors in esophageal cancer.

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